Recycling cellulase towards industrial application of enzyme treatment on hardwood kraft-based dissolving pulp

Qiang Wang^{a,b, c,*}, Shanshan Liu^{a,b, c}, Guihua Yang^a, Jiachuan Chen^a, Xingxiang Ji^{a,b}, Yonghao Ni^c

^a Key Lab of Paper Science and Technology of Ministry of Education, Qilu University of Technology, Jinan, Shandong Province, 250353, China

^b State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, Guangdong Province, 510640, China

^c Limerick Pulp and Paper Centre, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 5A3

ABSTRACT: Cost-effectiveness is vital for enzymatic treatment of dissolving pulp towards industrial application. The strategy of cellulase recycling with fresh cellulase addition was demonstrated in this work to activate the dissolving pulp, i.e. decreasing viscosity and increasing Fock reactivity. Results showed that 48.8-35.1% of cellulase activity can be recovered from the filtered liquor in five recycle rounds, which can be reused for enzymatic treatment of dissolving pulp. As a result, the recycling cellulase with addition fresh cellulase of 1 mg/g led to the pulp of viscosity 470 mL/g and Fock reactivity 80%, which is comparable with cellulase charge of 2 mg/g. Other pulp properties such as alpha-cellulose, alkaline solubility and molecular weight distribution were also determined. Additionally, a zero-release of recycling cellulase treatment was proposed to integrate into the dissolving pulp production process.

Keywords: Dissolving pulp; Cellulase treatment; Recycling; Cost effectiveness.

1. Introduction

Holistic using lignocellulose is the ideal of biorefinery concept (van Heiningen 2006). Pre-hydrolysis kraft-based dissolving pulp production process is a well commercial practice that fits well into the concept by separating hemicelluloses, lignin and cellululose into different streams (Saadatmand et al., 2013; Yang et al., 2013; Miao et al., 2014). The final product known as

dissolving pulp is used for various applications, such as viscose rayon and cellulose derivatives production (Jahan et al., 2011; Sixta et al., 2013; Tang et al., 2014; Iakovlev et al., 2014).

One of the main challenges of dissolving pulp production is the precise adjustment of viscosity and reactivity. Cellulase treatment is a near-commercial approach for decrease the viscosity and increase the reactivity in a gentle way (Engström et al., 2006; Gehmayr et al., 2011). The endoglucanase used for cellulase treatment preferably degrades amorphous cellulose located on the fiber surface and in-between the microfibrils, which increases the crystalline surface exposure and swelling ability and reactivity of the pulp (Ibarra et al., 2010). Wang et al. (2014) reported that the cellulase treatment increases the reactivity from 65% to 80% when upgrading pulp into dissolving pulp. Miao et al. (2014) observed the enhancement of accessibility and reactivity of dissolving pulp by cellulase treatment. However, the cellulase cost is still need to be reduced towards industrial application.

Cellulase recycling is a promising strategy to reduce enzyme cost. Cellulase, acting as a catalyst, will remain in the liquor phase after enzyme treatment of dissolving pulp. Recovery and reuse the liquor can not only reduce the enzyme cost, but also achieve the zero-release of water in the enzyme treatment stage. In fact, recycling cellulase has been investigated as early as 1971 in the bioconversion process of lignocellulose (Mandels et al., 1971). Subsequently, Lu et al. (2002) reported that 25- 83% cellulase protein can be recovered from the supernatant in the biomass hydrolysis process of pretreated softwood. Similarly, Tu and Saddler (2010) found that above 50% of cellulase specific activity can be recovered from liquor fraction. Furthermore, Wang et al. (2012) report that the various cellulase recyclability can be obtained regarding to the enzymatic substrate when studying the enzymatic saccharification for biofuel production.

The objective of this work is to evaluate the recycling cellulase from enzymatic treatment of dissolving pulp for viscosity and reactivity adjustment. The idea is to reduce the cellulase charge for producing desirable and stable pulp by reusing the recovered cellulase. Recycle rounds of five were performed to estimate the recycling enzyme treatment with/ without fresh cellulase addition. A process of recycling cellulase treatment was incorporated into the kraft-based dissolving pulp production process.

2. Materials and Methods

2.1 Materials

Bleached hardwood dissolving pulp was produced by a mill using pre-hydrolysis kraft-based production process in Eastern Canada, and the characteristics were alpha-cellulose content of 95.6%, intrinsic viscosity of 566.5 mL/g and Fock reactivity of 49.3%.

FiberCare D, a commercial endoglucanase-rich cellulase, was provided by Novozymes A/S (Denmark) and used as received. The cellulase activity is 35 000 U/mL determined by sodium carboxymethyl cellulose activity. All other chemicals used were of ACS reagent grade and purchased from Sigma-Aldrich. Deionized water was used for all the experiments.

2.2 Cellulase treatment

The cellulase treatment was carried out in plastic bag by using water bath. The treatment condition was pulp consistency of 3%, pH 4.8, 55 °C and 24 h according to previous study (Wang et al., 2015, Liu et al., 2015). Once the reaction was completed, the pulp was filtered and washed, then dried in a constant temperature-humidity room for a week for further analysis.

2.3 Recycle procedure

The recycle procedure was carried out by recycling the filtered liquor without wash water at the end of cellulase treatment. For each recycle round, the filtered liquor from the previous batch

was mixed thoroughly with fresh pulp. After treatment for 24 h, the liquor was collected from filter paper (Fisherbrand Filter Paper, P8). Additional fresh cellulase (0, 0.5, 1 and 1.5 mg/g) was added to the mixture. The process was then repeated for five recycle rounds.

2.4 Determination of recovered cellulase activity

Recovered cellulase activity was determined as sodium carboxymethyl cellulose activity. The cellulase treatment conditions as cellulase charge 10 mg/g pulp, pH 4.8, 55 °C and 24 h. Once the reaction was completed, the resultant liquor was filtered using syringe with a nylon membrane (0.45 µm pore size). Then, 0.3 mL of the filtered liquor was added into a 25 mL tube, which contained 2.0 mL of CMC-Na solution (pH 4.8, adjusted by sodium acetate-acetic acid buffer solution), and incubated at 55 °C for 30 min. After that, 3.0 mL of DNS reagent was added into the tube and boiled for 10 min. After that, the tube was cooled under tap water and diluted to 25.0 mL. The wavelength of 540 nm was used for the reducing sugar determination. Glucose was used as the standard.

2.5 Analytical methods for pulp

The determination of viscosity, Fock reactivity, S_{10} and S_{18} were conducted according to TAPPI T 230 om-94, modified Fock reactivity, and TAPPI T 235 cm-00. The molecular weight distribution of cellulose was determined by gel permeation chromatography (Waters 600E), equipped with a differential refractometer detector (Waters 410) and a Waters Styragel HT6E column according to previous studies (Wang et al., 2015). The mobile phase (0.5% LiCl/ DMAc) was pumped into the system at a flow rate of 1 mL/min; the system was operated at column temperature of 55 °C; the injection volume was 10 µL.

3. Results and Discussion

3.1 Recovered cellulase

The endoglucanase used in this study contains both cellulose binding module (CBM) and catalytic domain (CD). Initially, the CBM bound to cellulose to form cellulase-cellulose complex, and then the catalytic domain (CD) of cellulase randomly cleaved β -(1 $\rightarrow$ 4)-glucosidic bonds of cellulose to shorten cellulose chain. Desorption of cellulase from cellulose happened after cellulose chain cleavage.

The activity of recovered cellulase from enzymatic treatment of dissolving pulp after a series recycle rounds were determined. Then, the percentage of recovery activity was calculated as, 48.5% for 1st round, 42.9% for 2nd round, 40.5% for 3rd round, 38.1% for 4th round, and 35.1% for 5th round. As can be seen that about half of the initial cellulase activity was recovered in the first round, and then the decreased gradually. At the end of round 5th, one third of the initial cellulase activity remained in the filtered liquor. This is confirmed the recyclability of cellulase. The recovered cellulase activity has potential feasibility for reusing in enzyme treatment, which would greatly reduce the cellulase charge. Lindedam et al. (2013) reported that nearly all enzyme activity was lost in the supernatant for adsorbed to the residual biomass when studying enzymatic hydrolysis of pretreated wheat straw for cellulosic ethanol production. Whereas, Wang et al. (2012) concluded that the reversibility of cellulase binding to bleached pulp is higher than pretreated lignocellulosic substrate.

The effects of recycling cellulase treatment on viscosity and Fock reactivity of pulps are shown in Fig. 1. It is clearly shown that the viscosity decrease and Fock reactivity increase of round 1 was enhanced from the original pulp of 566.5 mL/g and 49.3%, which was great satisfied the industrial interesting for reusing the recovered liquor. By extending the recycle round, the enhancement extent was decreased, which is agreed with the decrease of cellulase

activity in the latter recycle rounds. By addition of small amount of fresh cellulase into the recycling liquor, the desirable pulp can be expected for the enzyme treatment.

3.2 Recycling cellulase treatment with fresh cellulase addition

Fig. 2 (a) and (b) showed the effect of fresh cellulase addition to the recycling cellulase treatment of dissolving pulp. As can be seen, the viscosity of treated pulps with recycling cellulase was further decreased for those added higher fresh cellulase under the same otherwise conditions. Interesting found is that the recycling treated pulps with fresh cellulase addition of 1 mg/g led to a similar viscosity as the non-recycling treated one with cellulase charge of 2 mg/g (Shown in Table 1). Correspondingly, the Fock reactivity of recycling cellulase treatment with addition of higher fresh cellulase was also enhancement compared with the control samples. Furthermore, the pulp properties kept constant in the five recycle rounds. 33-50% of cellulase charge can be reduced through recycling the liquor. Similarly, Tu and Saddler (2010) reported that the enzyme cost could reduce 24% when using surfactant to recycle the enzyme for bioconversion of pretreated softwood.

3.3 Comparison of recycling and fresh cellulase treatment of dissolving pulp

Table 1 summarized the properties of dissolving pulp from recycling cellulase and fresh cellulase treatment. As expected, the viscosity was decreased and Fock reactivity was increased after enzymatic treatment despite the recycling or fresh cellulase treatment. It is clearly showed that the recycling cellulase treatment yield pulp with lower viscosity and higher Fock reactivity under the same fresh cellulase charge of 1 mg/g.

The alkali solubility of dissolving pulp, i.e. S_{18} and $S_{10}-S_{18}$ represent the content of hemicelluloses and degraded cellulose, respectively. The S_{10} and $S_{10}-S_{18}$ of all treated pulps were slightly higher than the original pulp, indicating that the cellulase treatment degraded small

amount of cellulose, which also decreased the alpha-cellulose content. On the other hand, the recycling cellulase treatment with lower fresh cellulase addition produced dissolving pulp with higher alkali solubility. The 40-66% increase of alkaline solubility of dissolving pulp was also observed by Rahkamo et al. (1996) when using endoglucanase treatment of sulfite-based dissolving pulp.

The molecular weight of treated pulp was decreased from the control one, which further confirmed the cellulose chain cleavage. The molecular weight distribution of pulps was present in Fig. 3. The molecular weight distribution of treated pulp was shifted toward low molecular mass region. Similarly, Engström et al. (2006) observed the cellulose peak shifted towards the low molecular mass region after cellulase treatment when using Novozyme 476 to treat mixture of Norway spruce and Scots pine acid sulfite pulp.

3.4 Proposed process

The schematic of incorporating recycling cellulase into dissolving pulp production process was shown in Fig. 4. The key processes included: (1) recycling cellulase from the squeezed liquor, which reduced about 50% of cellulase dosage for desired pulp; (2) recirculating water from pulp washer, which achieved zero-release production process for enzymatic treatment. By recycling and reusing cellulase, the enzyme treatment showed great potential towards industrial application.

4. Conclusions

The strategy of recycling cellulase for enzyme treatment of dissolving pulp with fresh cellulase addition was demonstrated towards the industrial application. The recovered cellulase activity was in the range of 48.8-35.1% after five recycle rounds. The pulp treated by recycling cellulase and low fresh cellulase charge of 1 mg/g showed similar properties as the high fresh

cellulase charge of 2 mg/g. The determinations of alpha-cellulose, alkali solubility and molecular weight distribution were further supported the effectiveness of recycling cellulase treatment.

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227 **Figures Captions:**

228 **Fig. 1** Recycling cellulase treatment of dissolving pulp. Other conditions were: pulp
229 consistence of 3%, pH 4.8 and 55 °C.

230 **Fig. 2** Recycling cellulase treatment of dissolving pulp with fresh cellulase addition. (a)
231 viscosity, (b) Fock reactivity. Other conditions were: pulp consistence of 3%, pH 4.8 and 55 °C.

232 **Fig. 3** Molecular weight distribution of original and recycling cellulase treated pulps.

233 **Fig. 4** Schematic of recycling cellulase treatment and recirculation of process streams.

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Fig. 3 Molecular weight distribution of original and recycling cellulase treated pulps

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Fig. 4 Schematic of recycling cellulase treatment and recirculation of process streams

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Table 1 Comparison of dissolving pulps from recycling and fresh cellulase treatment.

Unit (mg/g pulp)	Original pulp	Recycling cellulase		Fresh cellulase	
		1 ^a	1.5 ^b	1	2
Viscosity (mL/g)	566.5	470. 2	448. 2	510. 0	472. .2
Fock reactivity (%)	49.3	80.0	85.5	74.3	85. 0
S ₁₀ (%)	5.50	7.87	8.14	6.58	9.5 4
S ₁₈ (%)	3.50	4.30	4.38	3.84	4.1 0
S ₁₀ -S ₁₈ (%)	2.00	3.57	3.76	2.74	5.4 4
Alpha- cellulose (%)	95.6	92.6	92.0	92.9	91. 8

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^a recycle round 1 with addition fresh cellulase charge of 1 mg/g,

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^b recycle round 1 with addition fresh cellulase charge of 1.5 mg/g.

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